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Analysis of the changes in PD−1/PD−L1 pathway function in non−small−cell lung cancer tissue before and after ^{125}I seed implantation

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ABSTRACT

Objective: To study the changes in PD-1/PD-L1 pathway function in non-small cell lung cancer tissue before and after ^{125}I seed implantation. **Methods:** Patients with advanced non-small cell lung cancer who were treated in Dongguan Kanghua Hospital between May 2014 and April 2017 were selected and randomly divided into ^{125}I group and control group who received ^{125}I seed implantation combined bronchial artery infusion chemotherapy embolism and bronchial artery infusion chemotherapy embolism alone respectively. The expression of PD-1, PD-L1, transcription factors and tumor suppressor genes in lung cancer lesions were determined before and after treatment. **Results:** PD-1, PD-L1 and Foxp3 mRNA expression and protein expression in lesions of both groups after treatment were significantly lower than those before treatment while T-bet, PTEN, TCF21 and LATS1 mRNA expression and protein expression were significantly higher than those before treatment, and PD-1, PD-L1 and Foxp3 mRNA expression and protein expression in lesions of ^{125}I group after treatment were significantly lower than those of control group while T-bet, PTEN, TCF21 and LATS1 mRNA expression and protein expression were significantly higher than those of control group. **Conclusion:** ^{125}I seed implantation treatment of advanced non-small cell lung cancer can inhibit the immune escape of cancer cells mediated by PD-1/PD-L1 pathway, and promote the apoptosis of cancer cells.

1. Introduction

Non-small cell lung cancer (NSCLC) is a pathological type of lung cancer with the highest incidence, early disease is relatively hidden and difficult to be diagnosed, and the majority of patients have developed to middle-advanced stage when diagnosed and cannot receive surgery. At present, systemic intravenous chemotherapy, radiotherapy and bronchial artery perfusion chemotherapy embolism are all used in advanced NSCLC therapy. Bronchial artery perfusion chemotherapy embolism is characterized by strong killing effect on cancer cells and low systemic side effects, and it is the ideal therapy for patients with advanced NSCLC[1,2]. However, the cancer cells will develop drug resistance during chemotherapy, which affects the effect of bronchial artery perfusion chemotherapy embolism. Radioactive ^{125}I seed implantation is the local radiotherapy developed in recent years, which persistently releases γ rays to achieve internal exposure to cancer cells and kill cancer cells[3,4]. The immune escape mediated by programmed death 1(PD-1)/PD-1 ligand (PD-L1) signaling pathway is the important pathogenesis of NSCLC, the changes in PD-1/PD-L1 pathway function in non-small cell lung cancer tissue before and after ^{125}I seed implantation were analyzed in the following study.

2. Information of lung cancer patients and research methods

2.1 General information of NSCLC patients

A total of 98 patients with advanced NSCLC who were treated in Dongguan Kanghua Hospital between May 2014 and April 2017 were selected as the research subjects, and all patients were with NSCLC confirmed by pathology, with TNM IIIa or IIIb stage and with expected survival time for more than six months. The patients who had been treated with radiotherapy and chemotherapy as well as targeted therapy, and the patients with radiotherapy and

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Table 1.
PD-1 and PD-L1 expression in lesions before and after treatment.

Groups	*n*	Time	mRNA expression		Protein expression	
			PD-1	PD-L1	PD-1	PD-L1
^{125}I group	49	Before treatment	1.04±0.15	1.09±0.18	1.86±0.25	3.25±0.62
		After treatment	0.36±0.07$^{*\&}$	0.31±0.06$^{*\&}$	0.64±0.09$^{*\&}$	1.42±0.18$^{*\&}$
Control group	49	Before treatment	1.07±0.19	1.05±0.16	1.91±0.26	3.31±0.69
		After treatment	0.68±0.09*	0.59±0.09*	1.21±0.18*	2.15±0.34*

*: comparison within group before and after treatment, $P<0.05$; $^{\&}$: comparison between 125I group and control group after treatment, $P<0.05$.

chemotherapy contraindications were excluded. The random number table was used to divide the enrolled 98 NSCLC patients into ^{125}I group and control group, each with 49 cases. ^{125}I group included 31 men and 18 women that were 45-62 years old; control group included 29 men and 20 women that were 43-63 years old. The comparison of general information showed no statistical difference between two groups of patients (P>0.05).

2.2 Research methods

2.2.1 Clinical therapy

Both groups of patients received bronchial artery perfusion chemotherapy embolism according to the following methods: the DSA machine of INTEGRIS Allura Monoplane was used for arteriography, the bronchial artery of the tumor blood supply was looked for in descending aorta tracheal bifurcation, micro catheter was selected for superselective catheterization, the intubation position was confirmed by angiography, 1 000 mg/m^2 of gemcitabine and 75 mg/m^2 cisplatin were injected, and then 300-500 μm and 560-710 μm of microsphere embolism particles and gelatin sponge were injected to make the tumor supply vessels occluded. ^{125}I group of patients received ^{125}I seed implantation according to the following method: CT scan was used to locate lesions and set the path for seed implantation, they received intramuscular injection of 5 mg morphine before implantation, then the implantation gun was used to implant seeds in accordance with the backward method, the seed spacing was 1 cm, and the distance to tumor edge was 0.5 cm.

2.2.2 Gene mRNA expression detection

Before treatment and 1 month after treatment, the right amount of lung cancer lesion was taken and added in Triol lysis buffer to extract RNA, reverse transcription kit was used to synthesize RNA into cDNA, fluorescence quantitative PCR kit was used to amplify cDNA, the amplified genes included PD-1, PD-L1, T-bet, Foxp3, PTEN, TCF21 and LATS1. The mRNA expressions of PD-1, PD-L1, T-bet, Foxp3, PTEN, TCF21, and LATS1 were calculated according to the amplification curve.

2.2.3 Gene protein expression detection

Before treatment and 1 month after treatment, the right amount of lung cancer lesion was taken and added in RIPA lysis buffer to extract total protein, BCA kit was used for determining the content of total protein, and enzyme-linked immunosorbent assay kit was used to detect PD-1, PD-L1, T-bet, Foxp3, PTEN, TCF21 and LATS1 contents. The protein expression of PD-1, PD-L1, T-bet, Foxp3, PTEN, TCF21 and LATS1 per mg total protein was calculated.

2.3. Statistical methods

SPSS 19.0 software was used to input and analyze data, gene expression data analysis between the two groups was by t test and P<0.05 indicated statistical significance in differences.

3. Results

3.1 PD–1 and PD–L1 expression in lesions of two groups of patients before and after treatment

Before treatment and 1 month after treatment, analysis of PD-1 and PD-L1 mRNA expression as well as PD-1 (ng/mg protein) and PD-L1 (ng/mg protein) protein expression in lesions between two groups of patients was as follows: PD-1 and PD-L1 mRNA expression and protein expression in lesions were not significantly different between two groups of patients before treatment (P>0.05); PD-1 and PD-L1 mRNA expression and protein expression in lesions of both groups after treatment were significantly lower than those before treatment (P<0.05), and PD-1 and PD-L1 mRNA expression and protein expression in lesions of ^{125}I group after treatment were significantly lower than those of control group (P<0.05).

3.2 Transcription factors T–bet and Foxp3 expression in lesions of two groups of patients before and after treatment

Before treatment and 1 month after treatment, analysis of transcription factors T-bet and Foxp3 mRNA expression as well as T-bet (ng/mg protein) and Foxp3 (pg/mg protein) protein expression in lesions between two groups of patients was as follows: T-bet and Foxp3 mRNA expression and protein expression in lesions were not significantly different between two groups of patients before treatment (P>0.05); T-bet mRNA expression and protein expression in lesions of both groups after treatment were significantly higher than those before treatment while Foxp3 mRNA expression and protein expression were significantly lower than those before treatment (P<0.05), and T-bet mRNA expression and protein

Table 2.
Transcription factors T-bet and Foxp3 expression in lesions before and after treatment.

Groups	*n*	Time	mRNA expression		Protein expression	
			T-bet	Foxp3	T-bet	Foxp3
^{125}I group	49	Before treatment	1.06±0.16	1.05±0.18	4.29±0.72	193.41±25.82
		After treatment	2.98±0.41$^{*\&}$	0.29±0.06$^{*\&}$	12.31±1.85$^{*\&}$	76.85±9.34$^{*\&}$
Control group	49	Before treatment	1.03±0.18	1.07±0.13	4.41±0.59	191.38±22.36
		After treatment	1.76±0.25*	0.56±0.09*	7.68±0.93*	125.68±16.48*

*: comparison within group before and after treatment, $P<0.05$; $^{\&}$: comparison between ^{125}I group and control group after treatment, $P<0.05$.

Table 3.
Tumor suppressor genes PTEN, TCF21 and LATS1 expression in lesions before and after treatment.

Groups	*n*	Time	mRNA expression			Protein expression		
			PTEN	TCF21	LATS1	PTEN	TCF21	LATS1
^{125}I group	49	Before treatment	1.02±0.16	0.98±0.11	1.05±0.15	2.48±0.35	94.51±11.25	1.56±0.19
		After treatment	3.25±0.52$^{*\&}$	2.80±0.39$^{*\&}$	2.57±0.32$^{*\&}$	7.59±0.93$^{*\&}$	278.65±35.51$^{*\&}$	4.89±0.62$^{*\&}$
Control group	49	Before treatment	0.95±0.12	1.04±0.17	1.02±0.14	2.54±0.37	95.11±10.28	1.60±0.20
		After treatment	1.84±0.26	1.67±0.22	1.70±0.25	4.47±0.61	146.75±20.37	2.84±0.33

*: comparison within group before and after treatment, $P<0.05$; $^{\&}$: comparison between ^{125}I group and control group after treatment, $P<0.05$.

expression in lesions of ^{125}I group after treatment were significantly higher than those of control group while Foxp3 mRNA expression and protein expression were significantly lower than those of control group ($P<0.05$).

3.3 Tumor suppressor genes PTEN, TCF21 and LATS1 expression in lesions of two groups of patients before and after treatment

Before treatment and 1 month after treatment, analysis of tumor suppressor genes PTEN, TCF21 and LATS1 mRNA expression as well as PTEN (ng/mg protein), TCF21(pg/mg protein) and LATS1(ng/mg protein) protein expression in lesions between two groups of patients was as follows: PTEN, TCF21 and LATS1 mRNA expression and protein expression in lesions were not significantly different between two groups of patients before treatment ($P>0.05$); PTEN, TCF21 and LATS1 mRNA expression and protein expression in lesions of both groups after treatment were significantly higher than those before treatment ($P<0.05$), and PTEN, TCF21 and LATS1 mRNA expression and protein expression in lesions of ^{125}I group after treatment were significantly higher than those of control group ($P<0.05$).

4. Discussion

Bronchial artery chemotherapy embolism is the common therapy for patients with advanced NSCLC, which can form high concentrations of chemotherapeutic drugs in local lesions and play a strong killing effect on cancer cells, and can also reduce the degree of the side reaction in normal tissue. In addition, the embolism of the tumor supply artery can cause cancer cell ischemia hypoxia, and then result in apoptosis and injury. Although bronchial artery chemotherapy embolism has achieved positive value for the treatment of patients with advanced NSCLC, there will still be different degrees of drug resistance in the process of chemotherapy and it will influence therapeutic effect. The ^{125}I seed implantation is a newly developed local radiotherapy method, which kills cancer cells with γ rays released by the ^{125}I seeds[5,6]. Excessive activation of PD-1/PD-L1 pathway is the important mechanism of the non-small cell lung cancer, and the signaling pathway can cause immunosuppressive tumor microenvironment, and make cancer cells escape from immunity and constantly proliferate and migrate, which lead to the growth of tumor lesion[7,8]. In order to define the killing effect of ^{125}I seed implantation on non-small cell lung cancer lesions, the PD-1/PD-L1 pathway function in lesions was analyzed before and after treatment in the study, and the results showed that PD-1 and PD-L1 expression in lesions of both groups after treatment were significantly lower than those before treatment, and PD-1 and PD-L1 expression in lesions of ^{125}I group after treatment were significantly lower than those of control group. This means that both bronchial artery perfusion chemotherapy embolism alone and ^{125}I seed implantation combined with bronchial arterial perfusion chemotherapy embolism can inhibit the PD-1/PD-L1 pathway function so as to inhibit the immune escape of cancer cells mediated by PD-1/PD-L1 pathway; the ^{125}I seed implantation combined with bronchial arterial perfusion chemotherapy embolism is more significant in inhibiting the PD-1/PD-L1 pathway than bronchial artery perfusion chemotherapy embolism alone.

PD-1 is mainly expressed on the surface of CD4+T cells, CD8+T cells, B cells, NK cells, mononuclear cells and other immune cells, belongs to the immunoglobulin B7-CD28 family, and contains intracellular segment, hydrophobic transmembrane area and extracellular segment. The intracellular segment of PD-1 contains the immune receptor tyrosine suppression sequence, and it can affect the differentiation of multiple T lymphocytes by combining with PD-L1[9,10]. The Th1 cell is an important subset in CD4+T lymphocytes that exerts anti-tumor immune response, and it can secrete cytokines such as IFN-γ and TNF-α to kill tumor cells; T-bet is a characteristic transcriptional factor on Th1 cell surface, which can regulate the differentiation and maturation of Th1 cells and the secretion of corresponding cytokines[11,12]. The Treg cell is the important subset in CD4+T lymphocytes that has immunosuppressive activity, which can significantly inhibit the anti-tumor immune response process; Foxp3 is a specific transcription factor for Treg cells, which regulates the differentiation

and maturation of the Treg cells[13,14]. The activation of the PD-1/PD-L1 pathway has an inhibitory effect on the differentiation of the Th1 cells and promotes the differentiation of the Treg cells. In order to define the effect of ^{125}I seed implantation on PD-1/PD-L1 pathway function in non-small cell lung cancer lesions, the immune cell transcription factor expression were further analyzed in the study, and the results showed that T-bet expression in lesions of both groups after treatment were significantly higher than those before treatment while Foxp3 expression were significantly lower than those before treatment, and T-bet expression in lesions of ^{125}I group after treatment were significantly higher than those of control group while Foxp3 expression were significantly lower than those of control group. That means that ^{125}I seed implantation combined with bronchial arterial perfusion chemotherapy embolism can more effectively promote the differentiation of Th1 cells and inhibit the differentiation of Treg cells so as to enhance the antitumor immune response and inhibit the immune escape of cancer cells.

In the process of immune escape of cancer cells, the expression of multiple suppressor genes is absent, which causes the abnormal proliferation of cancer cells. PTEN, TCF21, and LATS1 are tumor suppressor genes closely associated with NSCLC. PTEN-encoded products can reverse the phosphorylation of PI3K, which in turn inhibits the cell proliferation mediated by the PI3K/Akt pathway[15]; TCF21-encoded products can act on the KISS1 gene and trigger apoptosis[16]; LATS1-encoded products can interact with the HIPPO/YAP pathway to inhibit the cell proliferation and induce cell apoptosis[17,18]. In order to define the effect of ^{125}I seed implantation combined with bronchial arterial perfusion chemotherapy embolism on cancer cell proliferation and apoptosis in lesions, the expression of above tumor suppressor genes were analyzed in the study, and the results showed that PTEN, TCF21 and LATS1 expression in lesions of both groups after treatment were significantly higher than those before treatment, and PTEN, TCF21 and LATS1 expression in lesions of ^{125}I group after treatment were significantly higher than those of control group. This means that both bronchial artery perfusion chemotherapy embolism alone and ^{125}I seed implantation combined with bronchial arterial perfusion chemotherapy embolism can promote the expression of tumor suppressor genes and induce cancer cell apoptosis; ^{125}I seed implantation combined with bronchial arterial perfusion chemotherapy embolism is more significant in inducing tumor suppressor gene expression and cancer cell apoptosis than bronchial arterial perfusion chemotherapy embolism alone.

Based on above discussion, it can be concluded that ^{125}I seed implantation treatment of advanced non-small cell lung cancer can inhibit the immune escape of cancer cells mediated by PD-1/PD-L1 pathway, promote the differentiation of Th1 cells and inhibit the differentiation of Treg cells, and it can also induce tumor suppressor gene expression and promote cancer cell apoptosis.

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